Transgenic Beans with the Bean Golden Mosaic Geminivirus Coat Protein Gene are Susceptible to Virus Infection

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To improve bean production in Latin America, bean breeders need to continuously develop resistant varieties to the diverse populations of bean golden mosaic geminiviruses. Other control strategies rely on the application of new technologies like genetic engineering of beans with viral sequences that may confer a more stable and broad spectrum resistance. We report our evaluation of bean golden mosaic geminivirus (BGMV) coat protein-mediated protection strategy in navy beans (Seafarer) that were stably transformed by electric-discharge mediated particle acceleration (Russell *et al.*, 1993). This strategy is successful with several diverse plant RNA viruses (Kaniewski and Thomas, 1993).

In collaboration with University of Puerto Rico, we evaluated eight R_2 transgenic Seafarer lines having genes for BGMV coat protein (AV1), herbicide resistance (*bar*), and a color marker (*gus*). All transgenic lines had the AV1 gene and six out of eight expressed its mRNA (Table 1). However, none of the lines expressed coat protein by ELISA or Western blot analyses. Transgenic lines were challenge inoculated with the homologous virus by sap and whitefly transmission. Sap transmission results showed a BGMV incidence between 7 and 62%, and line 42-18 had the least virus incidence. Fig. 1 shows a Southern and a Northern blot for this line and other transgenic lines that had the viral gene and its mRNA before and after sap transmission. Although 42-18 line had a single copy of the AV1 gene integrated into its genome, as observed from the Southern blot, it expressed a high level of mRNA before BGMV challenge inoculation; and after inoculation, the level of mRNA decreased by three to five fold. Furthermore, the coat protein was not detected in this line either before or after challenge inoculation (Fig. 1, Western blot). When plants from the same line or the other seven lines were challenge inoculated by viruliferous whiteflies, BGMV incidence increased to 83-100%, and all lines, including line 42-18, exhibited severe symptoms similar to those of the nontransgenic plants. So, by whitefly inoculation, all transgenic beans were susceptible to BGMV.

The susceptibility of the transgenic lines to BGMV infection can be attributed to the failure of these lines to express the viral coat protein. Kunik *et al.* (1994) reported that there is a high correlation between the expression of coat protein and the expression of resistance in transgenic tomato lines that have the coat protein of tomato yellow leaf curl geminivirus (TYLCV). However, *Nicotiana benthamiana* transgenic lines, which expressed African cassava mosaic geminivirus (ACMV) coat protein, were susceptible when challenge inoculated with ACMV (Stanley *et al.*, personal communication). Since there is a discrepency between ACMV and BGMV vs TYLCV results, more bean lines should be evaluated for their expression of the viral coat protein and for their ability to confer resistance to BGMV. Because bean transformation continues to be difficult, our research team will focus on other antiviral strategies that have recently been successful for other whitefly-transmitted bipartite geminiviruses (Maxwell *et al.*, unpublished data).

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References: Kaniewski, W. K.,and Thomas, P. E. 1993. Seminars in Virology 4: 389-396.; Kunik *et al.* 1994. Bio/Technology 12: 500-504.; Russell *et al.*, 1993. Plant Cell Reports 12: 165-169.

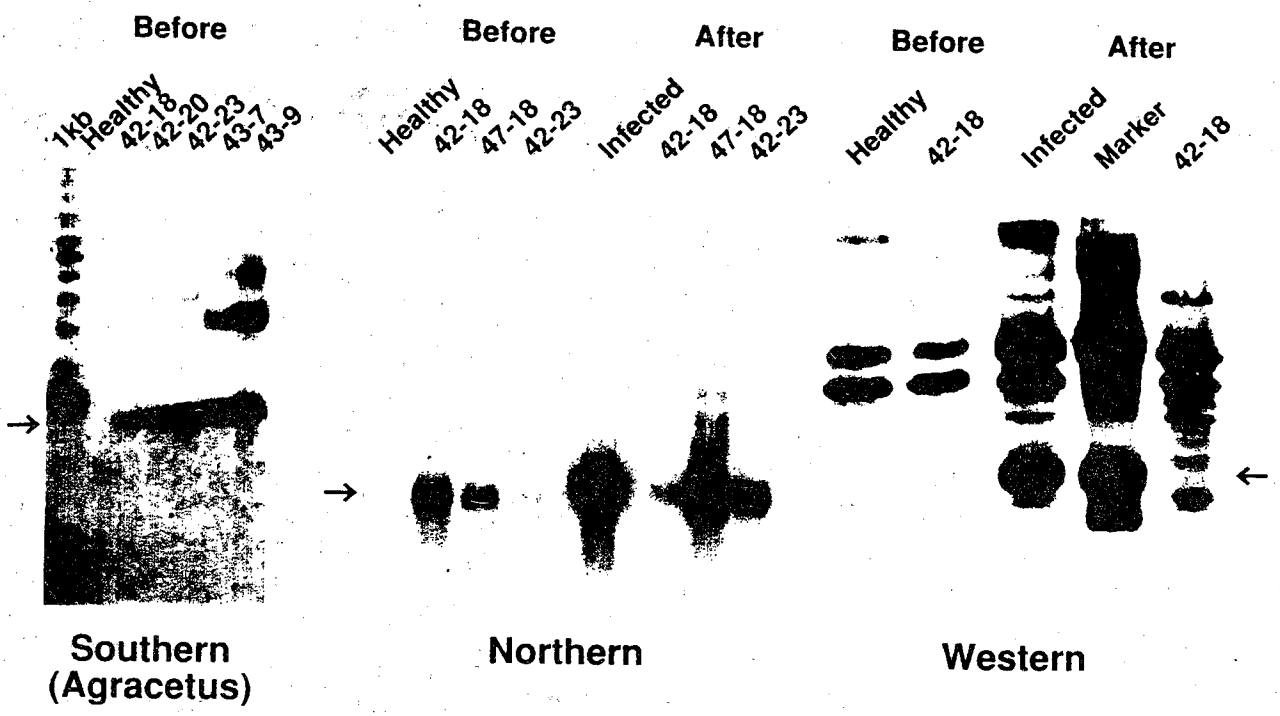

Fig. 1. Southern, Northern, and Western blots for detection of BGMV coat protein gene (AV1) and its products (mRNA and coat protein) in transgenic beans (Seafarer). Southern blot analysis of genomic DNA from nontransgenic plant (Healthy), lines 42-18, 42-20, 42-23, 43-7, and 43-9. DNA from individual R_1 plants was digested with *Cla*I and *Sma*I, transferred to a nylon membrane, and probed with radioactively labeled AV1 DNA. First lane shows the 1 kb marker. The arrow marks the expected size for the *Cla*I- *Sma*I fragment for AV1 ORF. Northern blot analysis was performed on total RNA extracts from a nontransgenic plant (Healthy and Infected lanes) and R_2 progeny plants of lines 42-18, 47-18, and 42-23 before and after sap transmission. Total RNA was transferred to a nylon membrane and probed with a radioactively labeled complementary sense transcript of AV1 DNA. Note the accumulation of mRNA in 42-18 line before and after BGMV challenge inoculation as compared to the accumulation of mRNA in other lines or in the nontransgenic control. The arrow marks the size of AV1 ORF. Western blot analysis of 42-18 line shows no accumulation of coat protein as compared to the accumulation of the coat protein from a nontransgenic plant before and after sap transmission. The arrow marks the size of the coat protein antigen.

Table 1. Reaction of eight R_2 transgenic bean lines to BGMV challenge inoculation by Sap and Whitefly transmission.

	Coat Protein			BGMV inoc./sap		BGMV inoc./whitefly	
	DNA	mRNA	Protein	Symp./inoc.	% Inf.	Symp./inoc.	% Inf.
Parent	-	-	-	13/19	68	24/24	100
Transgenic lines							
46-2	+	+	-	8/13	62	15/15	100
42-23	+	+	-	6/12	50	15/18	83
46-3	+	+	-	6/14	43	14/14	100
42-20	+	+	-	5/12	42	NT	-
43-3	+	-	-	6/15	40	21/21	100
43-9	+	-	-	4/13	31	21/21	100
47-1	+	+	-	3/13	23	21/21	100
42-18	+	+	-	1/14	7	32/32	100